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On the Genetics of Tetraploid Plants in Primula sinensis.

By R. P. Gregory, M.A., Fellow of St. John's College, Cambridge, University Lecturer in Botany.

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The purpose of this paper is to describe certain peculiar results obtained in the genetics of two "giant" races of *Primula sinensis.* Cytological investigation has shown these giants, unlike the giant races already described,* to be in the tetraploid condition, that is to say, that whereas in ordinary Primulas the chromosomes are x (12) in the gametic and $2x$ (24) in the somatic stage, in the tetraploid giants the chromosomes are $2x$ (24) in the gametic and, as nearly as can be counted, $4x$ (48) in the somatic cells.

Nilsson-Ehle† and East‡ have shown that factors of similar property may be reduplicated in the same zygote (or gamete), with various peculiar numerical consequences not otherwise intelligible, notably the appearance in certain F_2-families of such ratios as 15D : 1R, 63D : 1R, and so on, when in the ordinary case 3 : 1 would be expected. The occurrences to be described in part recall this phenomenon; but, as will be seen, they are

* Gregory, 'Camb. Phil. Soc. Proc.,' vol. 15, p. 239 (1909); Keeble, 'Journ. Genetics,' vol. 2, p. 163 (1912).

† "Kreuzungsuntersuchungen an Hafer und Weizen, I and II," 'Lunds Univ. Årsskrift,' 1909 and 1911; 'Berichte d. Deutschen Botanischen Gesellschaft,' vol. 29, p. 65 (1911).

‡ 'American Naturalist,' vol. 44, p. 65 (1910).

accompanied by others at first sight entirely paradoxical (as, for example, the fact that the ostensible recessive may throw the dominant), and the whole series may be regarded as of special significance in view of the association with the doubled condition of the cell-constituents. Moreover, in the tetraploid Primulas, the reduplication affects not merely the factors for isolated characters, but extends simultaneously to the factors for all the characters so far investigated.

The tetraploid giants with which I have worked are of two distinct races. One of these, which will be referred to as the GX race, consists of the progeny of a plant kindly given to me by Messrs. Sutton and Sons. The other race (GT race) arose in the course of my own experiments. Two non-giant diploid plants were crossed together reciprocally. The F_1 from one of these crosses gave a perfectly normal F_2, consisting of non-giant plants among which all the expected classes of offspring were represented in numbers closely approximating to expectation. The F_1 from the reciprocal cross gave no seeds in a cross with one of its parent races and gave only four plants as a result of self-fertilisation. These four plants were giants, and from one of them the GT race has been bred.

Up to the present time, neither the GX nor the GT races of giants have given any fertile seeds in crosses with various non-giant (diploid) races, whichever way the crosses were made. In this respect they differ from a diploid giant race, with which I have worked, which proved quite fertile with non-giants. It was this difference in behaviour which led to the discovery of the tetraploid nature of the GX and GT races.

In the tetraploid plants the chromosomes are naturally more crowded on the spindles than they are in diploid plants, but in polar views of the spindles of either of the maturation divisions there is no difficulty in determining that the number of chromosomes is normally 24 (compared with the 12 chromosomes found in diploid plants). In the somatic mitoses the chromosomes are longer than those of the maturation divisions and exact counts are difficult to make, but a number of counts have given numbers approximating to 48. The maturation divisions sometimes show some degree of irregularity, one or two chromosomes lagging behind the others in the movement to the poles, but I am not yet able to say whether fertile germ cells having more, or fewer, chromosomes than 24 are ever formed. There is no visible difference between the chromosome groups of the thrum-eyed (short-styled) and pin-eyed (long-styled) plants.

The two plants which were the progenitors respectively of the GX and GT races each possessed its own series of dominant characters, in respect of which its origin would indicate that it was heterozygous. In the

course of breeding in the direct line from these plants the recessive types have from time to time appeared. The course which this process of throwing recessives has taken is shown in the following table:—

Dominant character of parent.	Recessive character.	Generation in which the recessive first appeared.
	GX Race.	
Petals cut at the edges (*sinensis* type)	Petals heart-shaped with simple median notch (*stellata* variety)	F_3
Dominant white..................	Magenta flowers..................	F_4
Green stigma......................	Red stigma..........................	F_5
Magenta..............................	Red....................................	F_6
Palmate leaves	Fern leaves........................	F_6
	GT Race.	
Dominant white	Magenta............................	F_4
Magenta..............................	Red....................................	F_5
Thrum-eyed (short-styled) ...	Pin-eyed (long-styled)	F_6
Red stems	Green stems	F_6

In the character of the petals and in "dominant white" the dominance of the positive factor is not quite complete and the heterozygous plant can be distinguished from the pure dominant by inspection. In each of these cases, the appearance of the pure recessive is given in the table above, and in each case the heterozygote was recognised in the preceding generation.

In the GT race one expected recessive type, the double flower, has not yet appeared. But in F_3 two plants with semi-double flowers were obtained, both of which would no doubt have produced doubles among their offspring, had they not unfortunately succumbed to the attacks of fungus before they ripened seed.

It is obvious that some of the foregoing recessive characters have made only a belated appearance in the progeny of the original heterozygous plants. Both races of the tetraploid giants, however, produce a relatively very small quantity of seed in self-fertilisation, so that the families raised in each generation have nearly always been small. Consequently, in the present state of our knowledge of the processes of segregation in tetraploid plants, one cannot regard the sporadic appearances of the recessive types as providing a clear indication that processes other than the normal ones are involved.

Besides the recessive types, both races of giants have thrown some peculiar intermediate forms, which are distinct from any intermediate or other forms known to me in the non-giant diploid races. The characters, in

respect of which giant intermediates have been produced, include both morphological characters and colour-characters. They are—

	Dominant Character.	Recessive Character.
(1)	Petals cut at the edges.	Petals heart-shaped with simple median notch.
(2)	Tie-ring habit of the inflorescence.	Inflorescence condensed.
(3)	Palmate leaves.	Fern leaves.
(4)	Dominant white.	Coloured flowers.

During the present year there have also been obtained some flower-colours which are intermediate between magenta and red, but, as the diploid races also produce certain colours which it is difficult to classify, further experiment is necessary to show whether or not the new kinds of colour are peculiar to the tetraploid races.

With regard to the characters (1), (2), and (3) above, it should be pointed out that dominance is incomplete in the diploid races, but the giant intermediates form a distinct class from the common heterozygous type, which also occurs in the giant families, alongside of the peculiar intermediate types.

The intermediates between the palmate and fern leaves are, however, the most striking, because in the diploid races the dominance of the palmate shape is, for practical purposes, complete.

In all the cases there is some range of variation among the intermediate forms, and there may be differences of degree between the different organs of the same plant.

Further, it has been found that in the tetraploid giants certain types of flower-coloration may occur, which closely resemble the colours of certain diploid pure races, but are, nevertheless, the product of a different set of factors. This may be simply illustrated in the case of a Giant Red with red stigma, which almost exactly matched the colour of my Red *Stellata* non-giant race. The non-giant race is quite pure, and contains no colour-inhibiting factors. The giant red, selfed, has given (1) forms like itself, (2) more deeply coloured forms, and (3) pure and heterozygous "Duchess" types, that is to say, types showing the possession of the factor which inhibits the production of colour in the peripheral regions of the flower. Other similar cases have occurred, both in plants with green stigmas (*i.e.* possessing the factor which inhibits colour in the central parts of the flower), as well as in those with red stigmas. These cases, then, provide the striking result that the coloured form is shown to be capable of throwing the *dominant* white.

Phenomena of the kind just described, taken together with the fact that the tetraploid giants have produced intermediates peculiar to themselves, suggest some considerations as to the factorial constitution of the tetraploid plants. Both classes of phenomena can, I think, be explained by means of the hypothesis that, as compared with diploid plants, the tetraploid plants possess a double set of factors. Since in the zygote of a diploid pure race each factor is to be regarded as represented twice, AA, it follows that the tetraploid plant, according to this hypothesis, will be AAAA, and the gametes from which such a zygote is formed must be AA, that is to say, the factor will be represented twice in the gamete, instead of once, as it is in the gametes of the ordinary diploid race.

Heterozygous tetraploid plants may, then, be any one of three possible kinds, AAAa, AAaa, Aaaa. Since each gamete will contain two of the four units ("presences" or "absences") which make up the tetraploid group, the gametes produced by the three kinds of heterozygote, and the resulting progeny in F_2, will be as follows:—

Case I.—Heterozygote, AAAa; gametes, AA, Aa;
F_2, 1 AAAA : 2 AAAa : 1 AAaa.

No pure recessives in F_2, but, of every four plants, one will give pure recessives in F_3 in the proportion of one recessive in every 16 plants (see Case 2).

Case II.—Heterozygote, AAaa; gametes, AA, Aa, Aa, aa;
F_2, 1AAAA : 4 AAAa : 6 AAaa : 4 Aaaa : 1 aaaa.

F_2 contains one pure recessive in every 16 plants.

Case III.—Heterozygote, Aaaa; gametes, Aa, aa;
F_2, 1 AAaa ; 2 Aaaa : 1 aaaa.

F_2 contains one pure recessive in every three plants; no pure dominants, but one plant in every four will give pure dominants in F_3.

Of the various kinds of heterozygote shown in the foregoing scheme, one, namely AAaa, has the same proportion of positive and negative elements ("presences" and "absences") as the ordinary diploid heterozygote. With regard to the characters in respect of which the tetraploid giants have produced peculiar intermediates, it is suggested that the intermediates may be either AAAa or Aaaa. The former would presumably show the cumulative effect of the three factors, like that which Nilsson-Ehle and East have recognised in some of their cases, by giving a type more closely resembling the pure dominant than does the ordinary diploid heterozygote, but in the Primulas such types have not yet been definitely recognised by inspection.

Table I.—Crosses of Thrum-eyed and Pin-eyed Plants.

Thrum parent.	F_1.				F_1 plant.	$F_1 \times$ self.		$F_1 \times$ recessive.		
	Form of cross.	Index No.	Thrum.	Pin.		Thrum.	Pin.	Form of cross.	Thrum.	Pin.
$133^2/12$	$133^2/12$ as ♂	72/13	6	0	$72^3/13$	13	1	$72^3/13$ as ♀	3	4
					$72^5/13$	14	3	$72^5/13$ as ♀	15	19
					$72^6/13$	1	0	$72^6/13$ as ♀	6	12
	Ditto	86/13	18	1	$86^2/13$	7	0	$86^2/13$ as ♀	4	1
					$86^3/13$	22	1	$86^3/13$ as ♀	7	4
								Ditto	1	1
					$86^4/13$	19	8	$86^4/13$ as ♀	8	2
								$86^4/13$ as ♂	9	13
								Ditto	10	9
					$86^5/13$	4	4	$86^5/13$ as ♀	4	12
								Ditto	11	5
								$86^5/13$ as ♂	0	2
								Ditto	7	4
$133^3/12$	$133^3/12$ as ♂	87/13	15	1	$87^2/13$	28	10	$87^2/13$ as ♀	22	14
					$87^3/13$	27	10	$87^3/13$ as ♀	26	22
								$87^3/13$ as ♂	1	0
					$87^5/13$	8	3	$87^5/13$ as ♀	6	12
								Ditto	7	8
					$87^7/13$	7	1	$87^7/13$ as ♂	5	2
					$87^9/13$	30	10	$87^9/13$ as ♀	17	16
	Ditto	88/13	21	4	$88^2/13$	3	0	$88^2/13$ as ♀	7	0
					$88^3/13$	4	0			
					$88^4/13$	26	11	$88^4/13$ as ♀	46	38
								Ditto	3	9
					$88^6/13$	37	15	$88^6/13$ as ♀	26	25
								Ditto	4	2
					$88^{11}/13$	22	16	$88^{11}/13$ as ♀	5	1
								Ditto	23	18
					$88^{12}/13$	19	9	$88^{12}/13$ as ♀	6	1
								Ditto	14	4
					$88^{13}/13$	7	4	$88^{13}/13$ as ♀	2	0
								Ditto	1	2
								Ditto	1	1
					$88^{18}/13$	2	0	$88^{18}/13$ as ♀	9	9
$133^5/12$	$133^5/12$ as ♀	68/13	1	0	$68^1/13$	44	2	$68^1/13$ as ♀	2	1
								Ditto	13	3
								Ditto	1	0
								$68^1/13$ as ♂	51	14

The intermediate Aaaa would be expected to show the dominant character in less degree than the normal heterozygote; it is to this class that the intermediates already described are to be assigned.

The intermediate characters do not, however, provide the most favourable opportunity for putting the hypothesis with which we are dealing to a critical statistical test, because the range of variation among the intermediates is sufficient in some families to make classification by inspection a matter of difficulty. This difficulty will no doubt decrease as one becomes more familiar with the new forms, but for the present all that can be said is that the results of the experiments are in general accord with the present hypothesis.

A more critical test is, however, provided by some experiments relating to the characters of thrum-eye and pin-eye, and red stigma and green stigma. In these cases no intermediates have as yet occurred and it may be assumed that one "dose" of the factor is sufficient to bring about the development of the dominant character. The results of these experiments are set out in Tables I and II.

Table II.—Crosses of Green Stigma and Red Stigma.

F_1 plant.	$F_1 \times$ self.		$F_1 \times$ recessive.		
	Green.	Red.	Form of cross.	Green.	Red.
$72^3/13$	13	0	$72^3/13$ as ♀	5	2
$72^5/13$	17	0	$72^5/13$ as ♀	28	6
$72^6/13$	1	0	$72^6/13$ as ♀	14	4
$68^1/13$	44	2	$68^1/13$ as ♀	15	1
			Ditto	1	0
			Ditto	3	0
			$68^1/13$ as ♂	48	17

In these results two kinds of heterozygous F_1 plants are clearly shown to exist. Thus, in the crosses of thrum × pin (see Table I), the F_1 $68^1/13$ gave 41 thrum 1 pin when selfed, and 65 thrum 15 pin when crossed with the recessive. These numbers may, I think be regarded as representing respectively the ratios 15 : 1 and 3 : 1, and the F_1-plant may therefore be identified as AAaa. The thrum parents from which the other F_1's were obtained each gave a small number of recessives in the F_1-families from crosses with recessive plants. It is, therefore, not surprising to find that most of the F_1 thrum plants derived from their crosses have given F_2's approximating to the lower ratios 3 : 1 and 1 : 1; that is to say, the majority of the F_1's are of the constitution Aaaa.

To turn to the crosses of green × red stigma (Table II): it should first be pointed out that the GT race sprang from diploid races pure for green stigma and no red stigma has ever appeared in this race bred in the direct line. Plants of this race may, therefore, be written GGGG. It is entirely in accordance with this that the F_1's from crosses of this race with red stigma have all proved to be of the type GGgg, giving the ratios 15 : 1 when selfed, and 3 : 1 when crossed with the recessive. Heterozygotes of the type Gggg have, however, been found by selfing green-stigma plants chosen from families in which some of the plants had red stigmas. Ten such plants have given altogether 99 green stigma, 34 red stigma.

It will be noticed that the F_1's which appear in the green × red-stigma crosses also appear in the thrum × pin crosses. The F_1 $68^1/13$ is giving the same ratios in respect of each pair of characters, namely, 15 : 1 when selfed, and 3 : 1 when crossed by the recessive. But the F_1's 72/13 are giving 15 : 1 and 3 : 1 for green and red stigma, and 3 : 1 and 1 : 1 for thrum and pin. Taking the two pairs of characters together, and assuming for the moment that there are no special inter-relations between the factors, these would give the curious ratios of 45 TG : 15 tG : 3 Tg : 1 tg when the F_1 is selfed, and 3 : 3 : 1 : 1 when the F_1 is crossed by the double recessive (tg). The actual numbers obtained are 28 TG : 4 tG : 0 Tg : 0 tg in the former case, and 22 TG : 25 tG : 2 Tg : 10 tg in the latter.

In the foregoing results the different kinds of heterozygote stand out clearly identified by their progeny, and, although there are considerable discrepancies in individual cases, yet the general trend of these results clearly shows, I think, that the tetraploid plants are endowed with a double set of factors, as compared with the diploid races.

As has been remarked, the results of experiments with the intermediate types are in general accord with the idea that my existing intermediates are heterozygotes of the type Aaaa, in which one "dose" of the factor is not sufficient for the full development of the "dominant" character. The variations among the intermediates themselves, which are probably of the same nature as the variations exhibited among heterozygotes in cases where dominance is imperfect, are, of course, still to be explained. It is curious to notice that when there is any marked variation between the organs of the same plant it appears generally to take the form of a gradual retrogression towards the recessive character in the successively younger and younger organs, the effect of the positive factor being a little less pronounced in each new organ formed.

In conclusion, it must be remarked that the results so far obtained do not of themselves throw any direct light on the problem of the possible

relationships between the factors and the chromosomes. Although the fact that the duplication of the chromosomes has been accompanied by a duplication of the series of factors may seem at first sight to suggest a definite connection between chromosomes and factors, yet, on the other hand, the tetraploid number of chromosomes may be nothing more than an index of the quadruple nature of the cell as a whole. The case is, in fact, exactly analogous to the ordinary zygotic cell, which has $2x$ chromosomes and in which each factor is represented twice. But there are grounds for believing that further experiment with tetraploid plants may have a direct bearing in this connection, for some of the experiments have already given an unmistakable indication of the existence of special inter-relations (in the form of coupling or repulsion) between certain factors in the tetraploid Primulas. The work has not yet gone far enough to permit of any useful statement of the results, but it is obvious that it will provide a new opportunity for the study of the mutual relations between factors in heterozygous plants, particularly as to whether or not special inter-relations may occur between the two factors of the same kind (*i.e.* between A and A′), and as to whether either of the factors of one kind may have relations with either factor of another kind (*i.e.* A with either B or B′, and conversely), or whether the A and B factors form one pair of related factors, the A′ and B′ an independent pair, so that A may have special relations with B but none with B′, and conversely.

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